



Variability of manual ciliary muscle segmentation in optical coherence tomography images

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Abstract: Optical coherence tomography (OCT) offers new options for imaging the ciliary muscle allowing direct *in vivo* visualization. However, variation in image quality along the length of the muscle prevents accurate delineation and quantification of the muscle. Quantitative analyses of the muscle are accompanied by variability in segmentation between examiners and between sessions for the same examiner. In processes such as accommodation where changes in muscle thickness may be tens of microns- the equivalent of a small number of image pixels, differences in segmentation can influence the magnitude and potentially the direction of thickness change. A detailed analysis of variability in ciliary muscle thickness measurements was performed to serve as a benchmark for the extent of this variability in studies on the ciliary muscle. Variation between sessions and examiners were found to be insignificant but the magnitude of variation should be considered when interpreting ciliary muscle results.

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1. Introduction

The ciliary muscle has a key role in vision as one of the tissues involved in the biomechanical changes leading to accommodation. In the classical Helmholtz theory of accommodation, the muscle contracts and shifts anteriorly and inwardly, relaxing the zonular fibers connected to the crystalline lens, subsequently producing the change in lens shape essential to accommodation. Despite this important role, little is known about how the muscle and its contractability changes with age. In large part, studies of the muscle have been restricted due to the small size and location of the muscle behind the iris which prevent direct imaging of the muscle and muscle displacement *in vivo* [1]. Ultrasound [2–7] and MRI [6, 8] have been used to image the muscle. Ultrasound biomicroscopy (UBM) provides images of the muscle throughout its entire depth [2–7], but its resolution (0.1 mm) is insufficient to precisely quantify changes with accommodation, its contrast is limited, and it requires contact with the eye. MRI provides images of the entire ocular globe that show the anatomical relation between the muscle and other ocular structures [6, 8], but it has limited resolution (0.1–1mm), requires long imaging times, and is not adapted for routine use in a clinical setting due to cost of imaging and logistics. Recently, optical coherence tomography (OCT) has found increasing applications in ciliary muscle imaging, since the technique can provide direct visualization of the muscle through the conjunctiva and sclera. *In vivo* images of the muscle have been acquired with OCT not only at high resolution [9–12] but also dynamically during accommodation [9, 11].

Usage of OCT to study the ciliary muscle has raised questions regarding the accuracy of quantifying ciliary muscle dimensions from OCT images. Image quality has been observed to vary along the length of the muscle, notably due to reduced contrast at the apex of the muscle, its thickest portion [13–15]. The lack of definition in ciliary muscle boundaries complicates development of fully automated segmentation algorithms for the muscle [13]. Ciliary muscle boundaries near the apex of the muscle have low contrast and are therefore difficult to detect using edge detection, forcing most studies to rely on manual segmentation. Even with manual segmentation, low contrast of ciliary muscle boundaries adds uncertainty about muscle shape to examiners performing caliper measurements or manual segmentation. For ciliary muscle imaging where changes in the muscle are on the order of tens of microns during

accommodation [1, 9, 16, 17], a difference of a few pixels in segmentation can represent relatively large changes in the muscle, raising concerns about the reliability of manual measurements [18]. However, no extensive analyses of the effect of variability on ciliary muscle measurements from OCT images have been performed in the literature.

Understanding the extent of examiner variability in ciliary muscle measurements is needed to determine the contribution of examiner variability in measurements. In this study, we report intra- and inter-examiner variability of measurements of ciliary muscle thickness and thickness change during accommodation from two experienced examiners. The magnitude of variability determined will clarify changes in muscle due to physiology versus artifactual changes produced during quantification.

2. Methods

2.1 Study design

This study was approved by the Institutional Review Board at the University of Miami Miller School of Medicine and follows the tenets of the Declaration of Helsinki. All participants gave informed written consent. The left eyes of six young and healthy subjects (32 ± 9 y/o; range: 22 - 39 y/o) with no history of ocular disease were dynamically imaged in response to a step stimulus from 0 D (relaxed state) to 2 D (accommodated). Subjects' equivalent spherical refractive errors were -3.0 ± 3.6 D (range: + 1.50 D to -9 D).

2.2 OCT imaging system

A commercially available OCT (TELESTO, Thorlabs Inc., NJ) with 7.5 μm axial resolution, axial range of 2.5 mm (in air), and central wavelength of 1325 nm was used in ciliary muscle imaging. The ciliary muscle OCT (CM-OCT) is part of a previously reported OCT system [9] in combination with an anterior segment OCT (AS-OCT). The delivery probe of the CM-OCT is positioned to record the temporal side of the left eye and muscle through the conjunctiva and sclera. Only the temporal side can be imaged as the current optomechanical design of the system does not enable mounting of the ciliary muscle imaging probe on the nasal side.

The system also consists of an accommodation module which provides monocular step stimuli to the same eye that is imaged. The accommodation module combines two Badal optometers in a two channel configuration that enables presentation of either a far or near stimulus by switching the illumination of the respective channel on or off. To keep fixation constant during accommodation, the two channels were aligned to the optical axis of the eye [9]. The combination of the two OCT systems and accommodation module allows synchronous imaging of the ciliary muscle and anterior segment dynamically during the process of accommodation.

2.3 Imaging protocol

Subjects were seated with their heads stabilized by an ergonomic chin rest and head frame. Subjects were presented with the distance target, which was adjusted until the target was in focus while the near target was adjusted relative to the distance target to provide a 2 D stimulus. Before the start of the imaging session, near and distance targets were mutually aligned to the optical axis of the subjects' left eyes to enable steady fixation during stimulus presentation. The position of the CM-OCT was also adjusted to ensure the ciliary muscle was fully within view.

After alignment, subjects were asked to fixate on the distance target. OCT recording was started following which subjects were presented with an accommodative step stimulus after 1.5 s. Imaging was performed at 28,000 A-lines/s and 897 A-lines/frame with a lateral scan width of 8 mm, producing a dynamic set of 160 images over a duration of 6.2 s for the entire imaging session.

2.4 Image post-processing and data analysis

Each subject data set consisted of a single dynamic recording showing the response of the ciliary muscle from the relaxed to the accommodated state. Consecutive pairs of ciliary muscle OCT images from the data set were registered, averaged, and preprocessed to improve signal to noise ratio, resulting in a dynamic set of 80 images acquired at a rate of 13 frames/s. Tissue boundaries on the first ten images (when the muscle is relaxed) and last ten images (when the muscle is contracted) were manually segmented by two examiners who had prior experience segmenting the ciliary muscle in multiple subjects (Fig. 1). To perform segmentation, examiners utilized a custom software developed in MATLAB (MATLAB 2017a, The Mathworks Inc., MA). Examiners defined a boundary by selecting points along the boundary from the start and end of the boundary as judged by the examiner. Selected points were then connected through cubic interpolation to form a continuous boundary. To minimize the effect of interpolation on the performance of the segmentation examiners were instructed to use a similar number of points and consistent spacing between points for each boundary across images and across all subjects. Examiners were also instructed to use the smallest number of points that fully defined the boundary while keeping a smooth boundary which resulted typically in less than 10 points for the air-conjunctiva and sclera-ciliary muscle boundaries and less than 15 points for the more complex shape of the ciliary muscle-ciliary body boundary. The process produced smooth curves that followed the contour of the boundaries of the ciliary muscle.

For each subject, examiners completed a single tissue boundary starting from the first frame and proceeding to the next frame in time, finishing the first ten images then continuing to the last ten images in chronological order. After this tissue boundary was complete, examiners proceeded to draw the next tissue boundary in the same fashion, starting from the first frame and continuing to the next frame in time until the last image was reached. After segmentation of all three tissue boundaries (air-conjunctiva, sclera-ciliary muscle, and ciliary muscle-ciliary body) was completed once for the data set, the process was repeated an additional nine times. Upon completion of ten repetitions, examiners continued to the next subject until all subjects were processed.

Each segmented image was corrected for refractive distortion and optical path length using 1.415 and 1.380 for the group refractive indices of the conjunctiva/sclera and ciliary muscle, respectively [9]. The position of the scleral spur was determined as the average x- and y-location across the first ten and last ten images of the leftmost point of the sclera-ciliary muscle boundary. Ciliary muscle thickness (CMT) was calculated as the thickness at the inner apex (CMTMAX) and at other distances reported in literature, e.g. points at fixed distances from the scleral spur [1, 10, 14–16] and points proportional to the length of the muscle [1, 12]. Change in CMT was defined as the difference between the mean thickness of the last ten images (when the muscle is accommodated) and the first ten images (when the muscle is relaxed). In addition, relaxed and accommodated CMT were defined as the thicknesses in the first and last image of the data set, respectively.

To calculate intra-examiner variability, the standard deviation of the position of the scleral spur, relaxed and accommodated CMT, and CMT change across segmentation repetitions for a single subject was averaged across subjects and examiner. To calculate inter-examiner variability, the position of the scleral spur, relaxed and accommodated CMT, and CMT change were averaged across repetitions for every subject allowing comparison of subject measurements across examiners. Three-way repeated measures ANOVA were then performed (SPSS 24.0, IBM Corp., NY) to examine differences in intra- and inter-examiner variability across examiners, locations of thickness measurements, and thicknesses and thickness change.

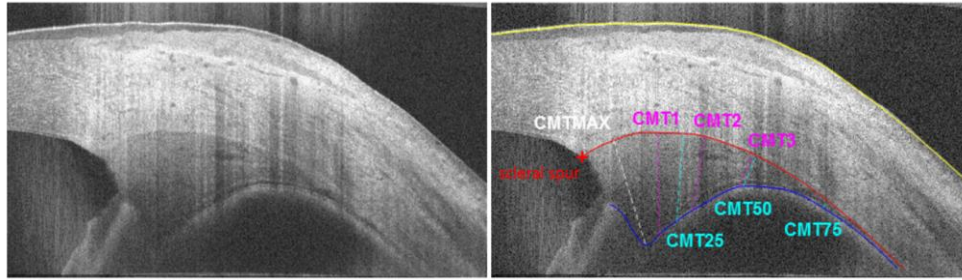


Fig. 1. *Left* Ciliary muscle OCT image. *Right* Example of manual segmentation (yellow, red, and blue lines). Ciliary muscle thicknesses are highlighted by dashed lines, showing a white dashed line for the maximum thickness, magenta dashed lines for thicknesses at 1, 2, and 3 mm from the scleral spur along the length of the outer boundary of the muscle, and cyan dashed lines for thicknesses at 25, 50, and 75% of the length of the outer boundary of the muscle from the spur, which is indicated by a red cross. Aspect ratio of images have been adjusted for publication.

3. Results

3.1 Intra- and inter-examiner variability in selecting scleral spur

Intra-examiner variability in the position of the scleral spur did not differ significantly between examiners ($p = 0.92$) with a variability of 3.0 pixels, corresponding to $29 \mu\text{m}$. Similarly, inter-examiner variability did not attain statistical significance ($p = 0.37$) with an average difference of 11.6 pixels, corresponding to $122 \mu\text{m}$ between examiners.

3.2 Intra-examiner variability in measuring ciliary muscle thickness

An example of intra-examiner variability for a single subject is shown in Fig. 2. Intra-examiner variability was calculated for measurements at and between different accommodative states and at different locations along the ciliary muscle and then averaged across subjects. Differences in intra-examiner variability were not significant across examiners ($p = 0.33$), and results after averaging across examiners are shown in Table 1. Differences in intra-examiner variability were also not significant across measurements at different locations along the ciliary muscle ($p = 0.42$) and between thickness and thickness change measurements ($p = 0.11$). Average intra-examiner variability across subjects and examiners was $9 \mu\text{m}$.

Table 1. Intra-examiner variability for examiners A and B and for relaxed and accommodated ciliary muscle thickness measurements, and accommodative ciliary muscle thickness change measurements at different lengths along the muscle. Intra-examiner variability is shown as the standard deviation of measurements over ten repetitions averaged across subjects and examiners.

CMT (μm)	Relaxed CM		Accommodated CM		Change b/t Accommodated and Relaxed CM	
	Examiner A	Examiner B	Examiner A	Examiner B	Examiner A	Examiner B
CMTMAX	8	7	10	9	12	9
CMT1	9	8	11	9	9	9
CMT2	6	7	8	9	9	7
CMT3	6	5	7	5	7	4
CMT25	10	9	14	9	12	9
CMT50	13	7	15	7	14	6
CMT75	12	6	13	6	12	6

3.3 Inter-examiner variability in measuring ciliary muscle thickness

Inter-examiner variability was calculated for measurements at and between different accommodative states and at different locations along the ciliary muscle and then averaged across subjects. As shown in Table 2, ciliary muscle thicknesses decrease in both the relaxed and accommodated state at locations further away from the scleral spur. Changes in muscle thickness due to accommodation decreased along the length of the muscle, reflecting a shift of muscle mass more anteriorly. In addition, inter-examiner variability was not significant across examiners ($p = 0.16$). Agreement between examiners is depicted in Bland-Altman plots shown in Fig. 3.

Table 2. Inter-examiner variability for examiners A and B and for relaxed and accommodated ciliary muscle thickness measurements, and accommodative ciliary muscle thickness change measurements at different lengths along the muscle. Inter-examiner variability is shown as measurements for each examiner after averaging across repetitions and subjects.

CMT (μm)	Relaxed CM		Accommodated CM		Change b/t Accommodated and Relaxed CM	
	Examiner A	Examiner B	Examiner A	Examiner B	Examiner A	Examiner B
CMTMAX	532	552	563	588	31	35
CMT1	463	488	480	503	18	14
CMT2	317	341	304	345	-13	4
CMT3	180	203	170	194	-10	-8
CMT25	412	417	421	434	10	17
CMT50	203	202	190	191	-13	-11
CMT75	91	96	88	99	-3	2

4. Discussion

Intra- and inter-examiner variability of ciliary muscle thickness and thickness change measurements during accommodation derived from OCT images was reported. Differences in both intra-examiner and inter-examiner variability were not statistically significant with an intra-examiner variability of 9 μm and average absolute difference of 13 μm between the two examiners. Given accommodative thickness changes of 17 $\mu\text{m}/\text{D}$ for CMTMAX and less than 8 $\mu\text{m}/\text{D}$ in absolute value for the other positions along the muscle boundary, the intra- and inter-examiner variability is sufficient to detect change in the muscle in response to incremental accommodation in steps of 1 D near the apex with manual segmentation. At the other locations, higher precision is required to allow measurements of changes in response to incremental accommodation steps. The results suggest that manual segmentation is reliable for determining ciliary muscle measurements. In general, muscle thickness in both the relaxed and accommodated state decreased further away from the scleral spur. In addition, accommodative changes in ciliary muscle thickness reported in this study showed trends consistent with prior research [1, 12, 14], where accommodative changes closer to the apex of the muscle were larger than those in parts more posterior to the muscle.

However, key differences exist in intra-examiner variability between ciliary muscle thickness and thickness change measurements. Although thickness change measurements are derived from subtraction of accommodated and relaxed thicknesses and should have a variability of approximately that of the thicknesses combined, intra-examiner variability was not significantly different between thickness and thickness change measurements. This suggests that the variability is due in part to differences in where examiners visualize the boundary of the muscle. As examiners segment a dynamic data set, their memory of where the boundary was placed in relaxed muscle images may influence placement of the boundary in accommodated muscle images, such that a bias is maintained from processing relaxed to

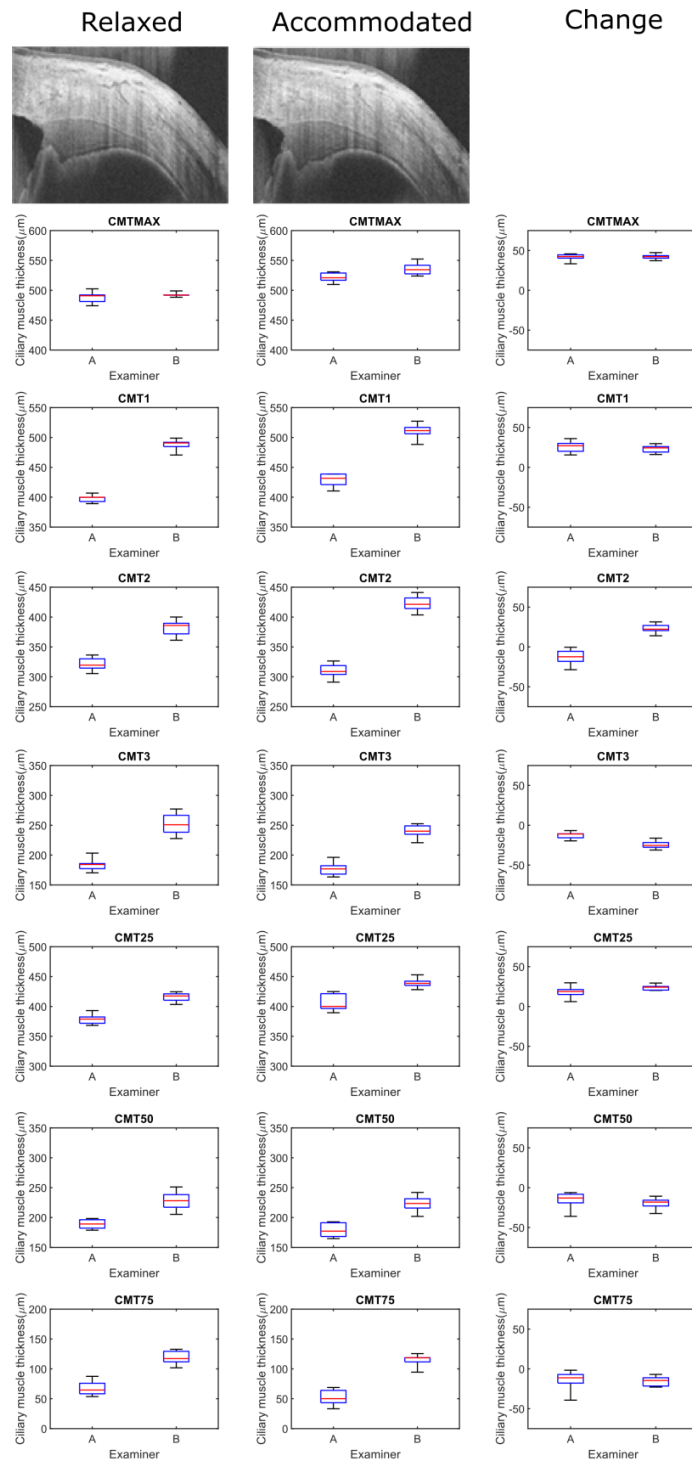


Fig. 2. (Top row) OCT images of the relaxed and accommodated ciliary muscle in a sample subject. (Lower rows) Boxplots show variability of ciliary muscle relaxed and accommodated thickness measurements, and accommodative thickness change measurements over 10 repetitions of manual segmentation of the muscle for each examiner and for various distances along the length of the muscle.

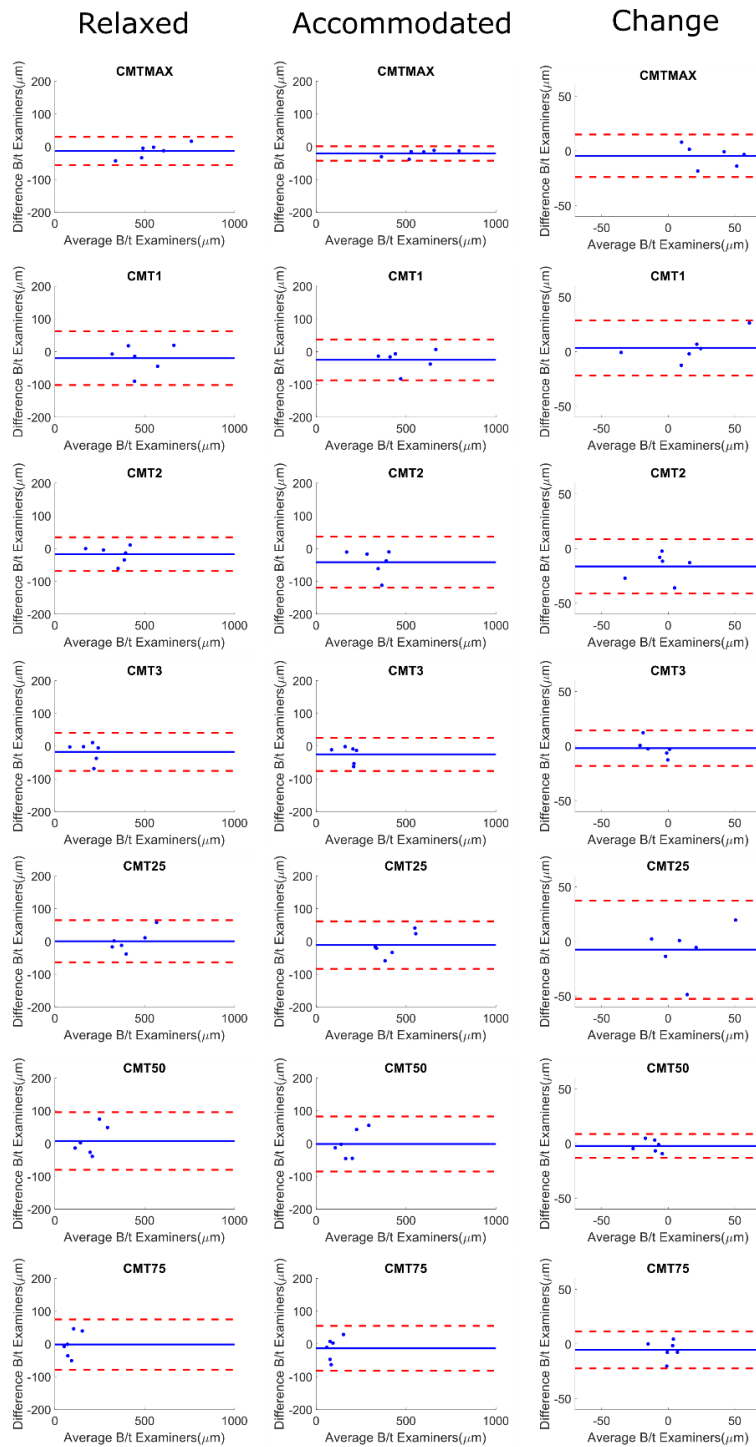


Fig. 3. Bland-Altman plots showing agreement between Examiners A and B for relaxed and accommodated ciliary muscle thickness measurements and accommodative thickness change measurements at different locations along the ciliary muscle. Muscle thicknesses at different locations are highlighted with different colors while mean and limits of agreement are shown with solid and dotted lines, respectively.

accommodated ciliary muscle images reducing variability in thickness change measurements. To avoid potential memory bias, manual segmentation can be performed in a blind randomized fashion where examiners are asked to segment images that are randomly selected from a population of subjects. Examiners would have to wait until all of the data is collected, and a large database would be needed to truly avoid the effect of memory. Such an approach is not carried out in practice as processing of data from individual subjects likely occurs after data acquisition for the subject and for all repetitions of the subject before moving on to the next subject. We therefore recommend that multiple observers process images to minimize the effect of memory bias. Also of note, differences in intra-examiner variability at different locations along the ciliary muscle were not significant. Although previous studies noted reductions in image contrast towards the apex of the muscle [13–15], the reliability of manual segmentation remains consistent throughout the length of the muscle. Similarly, there has been debate regarding where on the muscle to measure muscle thickness [18]. Using locations based on fixed or percent distances may introduce errors due to the lack of clear landmarks for the posterior part of the muscle and the difficulty of identifying the scleral spur. Given the lack of significant differences in intra-examiner variability across different muscle locations, the result suggests the variability of using locations based on fixed or percent distances is largely dependent on variability inherent to examiners rather than variability due to the difficulty of finding the same location across images. This conclusion is echoed by the small variability seen in selection of the scleral spur within and between examiners (3.0 pixels and 11.6 pixels, respectively).

Certainly, images from different subjects may vary in image quality whereby the definition of tissue boundaries may be readily apparent in some compared to others. To provide an accurate depiction of variability, subjects were chosen who had ciliary muscle images where muscle tissue were visible up to the muscle apex. Similarly, different OCT imaging systems and circumstances may lead to differences in the magnitude of variability in segmentation among studies. In comparison with other OCT studies reporting variability of ciliary muscle measurements, the magnitude of intra-examiner variability in the present study is similar to that of Sheppard et al [1], whereas the intra- and inter-examiner variability is tens of microns lower than the reported values in Kao et al [15]. A possible explanation for our lower values may be that variability analyses were performed on dynamic data sets acquired with a specific commercial OCT system. The dynamic nature of measurements minimizes the potential for changes in alignment during imaging, reducing variability from changes in the location of measurements rather than variability from segmentation. Kao et al acknowledged that changes in alignment between imaging sessions may have added to the intra-examiner variability of their measurements. In addition, the present OCT system provides greater contrast of the ciliary muscle than the clinical systems normally used for ciliary muscle study [1, 14] and a recently reported custom-built OCT system [17], allowing the present OCT system to potentially help examiners discern the boundaries of the ciliary muscle more accurately [13].

Finally, ciliary muscle measurements were calculated from manual segmentation of ciliary muscle boundaries, whereas in prior studies, measurements were typically derived from manual placement of calipers on singular points along ciliary muscle boundaries. When using calipers, ciliary muscle locations (e.g., CMT1, CMT2, etc.) are not measured from the scleral spur along the contour of the muscle as performed in this study but rather measured along a straight line drawn from the scleral spur to a point on the posterior sclera-muscle boundary. As mentioned by Kao et al, a line from the scleral spur to the posterior part of the muscle does not accurately represent the curvature of the sclera-muscle boundary, leading to measurements in arbitrary locations along the muscle. With entire muscle boundaries, measurements can be more accurately derived especially given that the signal at single points along the ciliary muscle may be subject to noise or differences in contrast. In light of the steps

taken in present study to isolate variability due to segmentation alone, the results serve as a “best case scenario” of variability in ciliary muscle measurements.

Other possible concerns involve the use of only one stimulus level for studying variability in the accommodated ciliary muscle. We provided only a 2 D stimulus because the goal of the study was to evaluate if the system had sufficient sensitivity to measure small changes in accommodation in a repeatable fashion. Subjects, particularly those with high myopia, can demonstrate accommodative lag in response to small stimuli. However, the use of a small stimulus and potential variability in accommodative response was not a major concern in this study since the goal was to quantify the intra- and inter-examiner variability on each individual subject. In addition, it may be that variability may change with accommodation as prior studies noted greater difficulty in visualizing the apex in the accommodated muscle in contrast to the relaxed muscle [14, 15]. Intra-examiner variability did not show significant differences between measurements on the relaxed and accommodated muscle. Thus, accommodation does not appear to affect variability of ciliary muscle measurements, and additional stimulus levels would prove repetitive.

Intra- and inter-examiner variability in ciliary muscle measurements derived from manual segmentation of OCT images were determined to be similar across examiners supporting the reliability of manual segmentation. Conclusions based on ciliary muscle measurements from OCT studies should account for this level of variability when differentiating between physiological changes and changes produced by variability in manual segmentation.

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Disclosures

Authors have no relevant disclosures.